Clinical applications of MR spectroscopy in oncology

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Introduction

One of the first applications of Magnetic Resonance Spectroscopy (MRS) to a patient with a tumor was the detection of an abnormal ³¹P MR spectrum of a rhabdosarcoma as compared to that of muscle tissue [1]. A typical tumor feature, already evident in this spectrum, was the relatively high level of the so-called phosphomonoester peak, which contains resonance's from phosphorylated choline and ethanolamine [2]. Later when ¹H MRS was applied to patients with brain tumors a relatively high signal was observed for a peak at about 3.2 ppm [3]. This peak turned out to be mainly composed of the resonances from methyl groups of "choline compounds" such as choline, phosphocholine and glycerophophocholine of which the ¹H chemical shift dispersion is too small to be resolved in the in vivo spectrum. Further MRS studies of other human tumors also revealed the presence of relatively high levels of choline compounds indicating this to be a general neoplastic phenomenon, which attracted much attention as it could serve as a potential biomarker in cancer diagnosis (detection, grading and staging) and treatment response [2, 4]. The choline compounds are involved in the biosynthesis and degradation of phospholipids such as phosphatidylcholine, which is required for the build-up and maintenance of cell membranes. Choline compounds are differentially increased in tumors depending on the type and grade of the tumor. The exact reason behind this increase is still unclear but may be caused by increased choline transport into tumor cells, increased choline kinase activity and increased phospholipase expression and activity in tumors [4-6]. The composite peak at about 3.2 ppm in the ¹H MRS spectrum is commonly referred to as the total choline peak (tCho), although it may also contain (smaller) contributions from other compounds (e.g. taurine or myo-inositol).

Next to the characteristic content in tCho, MRS of human tumors has uncovered abnormal tissue levels of many other metabolites reflecting changes in metabolism, morphology or physiology due to neoplastic growth. Several of these metabolites are specific for the tissue from which the tumor originates, but abnormal levels of the individual compounds are generally not specific for tumor growth only and may occur in other pathologies. However, a typical composition of several metabolite resonances in a metabolic profile may be characteristic for a particular tumor growth.

In clinical applications of MRS the ¹H nucleus has played a dominant role, mainly because this nucleus is present in most body metabolites and can be detected with high sensitivity. In addition protons can be employed relatively easy on common clinical MRI machines, which are dedicated to the detection of protons in water and fat. However, other MR sensitive nuclei such as ³¹P, ¹⁹F and ²³Na may provide highly relevant and unique information on tumor metabolism and physiology, but their application in a clinical setting has been limited up till now. As higher field MR systems are being introduced in the clinic now it may become more attractive to make use of these nuclei in clinical examinations of tumors. Although interesting clinical results have been obtained in human tumor studies using other nuclei, e.g. ¹⁹F [7]and ³¹P[8], we restrict ourselves below to a brief summary of the main clinical applications of MRS to oncological problems using the ¹H nucleus: i.e. in the brain, prostate and breast.

Robust acquisition methods to obtain localized ¹H MR spectra from single and multiple volumes are already available since the 1990's for clinical applications in the brain. But only recently such methods are becoming available for prostate and breast. A thorough description of the technical details of acquisition methods is beyond the scope of this lecture but some particular aspects will be discussed below. Further progress in MRS technology applicable to tumors is ongoing, for instance parallel acquisition methods and more automation in data acquisition and processing. In addition new methods are implemented at 3T, to explore the potentially better signal to noise and chemical shift dispersion as compared to 1.5T systems. And recently new ways are being explored for sensitivity enhancement with hyperpolarization [9]. A particular advantage of MR spectroscopy is that it provides

quantitative data, which is not common practice in the clinical assessment of tumors by most (conventional) MRI methods. A general trend nowadays is to combine MRS data with that of other MR approaches such as conventional T1 and T2 weighted MRI, dynamic contrast MRI, and diffusion MRI as it may improve the diagnostic performance beyond that of each single MR approach.

In vivo ¹H MRS of brain tumors

The histopathological examination of biopsy material is the decisive step in the diagnosis of a brain tumor to establish type and grade, which in turn determines which further treatment procedures are followed. Although biopsy material is commonly available there are multiple cases where an adequate non-invasive assessment would be desirable to avoid a biopsy: e.g. in cases with difficulties in the differential diagnosis with non-neoplastic diseases or with high risks of morbidity or mortality. Radiological examination by MRI, including Gadolinium contrast application, is an important element in the work-up of patients suspected of a brain tumor. ¹H MRS is now acknowledged as a useful complement to MRI in the management of brain tumors as it provides unique metabolic information that can be diagnostic for tumor type and grade, and may serve as a predictive tool, in the planning and evaluation of treatments, e.g. to identify recurrence as compared to radiation necrosis [10, 11].

Single voxel ¹H MRS is the easiest and quickest way to obtain metabolic information of tumor tissue in the human brain. Commonly localized MR spectra are obtained by a STEAM or PRESS pulse sequences at long echo time (about 270 ms), intermediate echo time (about 135 ms) or short echo time (about 20 – 30 ms or less). At longer echo times less metabolites are visible, but as the resonances are less cluttered data processing is easier. To measure viable tumor tissue the voxel is positioned by MRI guidance, e.g. on the Gd enhancing part of the tumor. To assess the heterogeneous nature of tumors in the brain a multiple voxel method is needed, for which usually a large brain volume is selected also covering non-tumor tissue, which is divided up in smaller voxels by phase encoding methods.

The main metabolites visible in a ¹H MR spectrum of the human brain obtained at intermediate or long echo times are N-Acetylaspartate (NAA) and total creatine (tCr) and choline (tCho) compounds. Most characteristic for tumor tissue in the brain is an increase in the tCho level as compared to unaffected tissue. This level appears to be correlated with the malignancy of the tumors as expressed in proliferation activity, grade or cellular density [11-15], but as yet overlap in the data precludes individual assignments based on the level of this peak only. The tCr content is decreased in some brain tumors and as creatine is an important compound in energy metabolism it is assumed this indicates abnormal energy metabolism. A reduction in NAA is a general observation in adult brain tumors. This reflects the glial origin of most tumors as NAA predominantly occurs in neurons, which are replaced by the tumor tissue. NAA reduction is a rather non-specific observation as it also occurs in other brain pathologies. At intermediate or long echo times a doublet signal of lactate or of alanine may be observed for tumor tissue. The observation of lactate initially raised much interest, as lactate is an end product of aerobic glycolysis, which is an important trait of more aggressive tumors. However, a lactate signal may also be seen because it is not cleared from tumor tissue due to accumulation in necrotic or cystic regions, which is not necessarily associated with increased glycolysis [13, 16]. The observation of an alanine signal appears to be characteristic for meningiomas [17, 18].

At shorter echo times additional signals become visible in the spectrum of brain tissue that may also have diagnostic potential. For instance, the particular levels of compounds such as myoinositol, gluthathione, glutamate and glutamine may help to differentiate low and high grade gliomas [19, 20], astrocytomas, schwannomas, and hemangio-pericytomas from meningiomas [18, 21-23], and oligodendriogliomas from astrocytomas [24]. The latter is of interest as oligodendriogliomas may better respond to chemotherapy.

Lipid and macromolecular signals are of special interest in the diagnosis of brain tumors as high intensity of these signals are very characteristic of high-grade tumors e.g. [11, 25], which may be related to membrane breakdown and (micro-)necrosis [26], or intracellular triglyceride droplets [27]. The specific profile of lipids may help to differentiate glioblastomas from metastases, which otherwise have comparable spectra [28].

Before refining the diagnosis of a brain tumor in a patient, a differential diagnosis between neoplastic and non-neoplastic growth may be needed first. Charactistic features such as increased tCho and decreased NAA may be used for this purpose but this has to be done with caution as some pathologies such as reactive gliosis or sclerosis may show typical tumor-like MR spectra, especially

like low grade tumors e.g. [29, 30]. More advanced data acquisition and processing may improve the specificity of MR spectroscopy to differentiate tumor from non-tumor cases e.g. [31]. Certain brain pathologies may be easily differentiated as they show MR spectra that are very different from those typical for tumors, for instance those of abscesses [32, 33].

MR spectroscopy can help in stereotactic procedures to obtain biopsies from the proper tumor locations, i.e. the most malignant parts [34, 35] or to delineate the tumor lesion for planning of surgery or radiation treatment. [36-39]. In infiltrating tumors like glioblastomas MRS may identify tumor load outside the Gd enhancing area, which makes MRS especially useful in treatment planning next to other MR modalities. In all these cases the use of 3D multivoxel MRS approaches is essential. The tCho signal is most often used as marker to assess response to therapy of brain tumors by MR spectroscopy [11]. Of particular clinical interest in treatment follow-up of brain tumors is the discrimination of tumor recurrence from radiation necrosis by MRS, as there are little other ways to asses this properly [40-44].

The added diagnostic benefit of ¹H MR spectroscopy to conventional MRI has been demonstrated in a few studies e.g. [21, 45-47]. The assessment of brain tumors in children by MRS have not been explicitly described here but pediatric cases have to be considered as a separate group with specific clinical and diagnostic needs, e.g. see [29, 48-50].

As the analysis of MRS data of brain tumors may be rather complex to less experienced users in clinical routine and also because the proper analysis of large datasets from multi-voxel assessments is unpractical, much attention has been given to automated and objective processing and classifying of voxels of such data by a variety of approaches e.g. [32, 37, 47, 51-58]. To develop reliable and clinically useful classifiers of tumor type and grade it is necessary to have a sufficiently large dataset available of these tumors, acquired with some flexibility in data acquisition modes, but with proper system, spectral, clinical and histopathological quality control. This has been accomplished in the multi-center EU project INTERPRET [55, 59] and this work is currently expanded in a new project eTUMOUR, also including other modalities to assess brain tumors [60].

In 2003 two reports appeared of studies using evidence based medicine (EBM) criteria to evaluate the diagnostic and therapy decision-making value of MRS applied to patients suspected to have a brain tumor, based on available literature. Today only one is retrievable from the internet[61]. The essential conclusion was that, despite numerous publications showing the usefulness of MRS examinations of human brain tumors, too little studies have focused on a proper evaluation of the clinical value of MRS using standards common in EBM, and hence no clinical value could be demonstrated. The EBM reports have been critically discussed recently [62, 63]. Whatever the quality of these reports may be, it is clear that carefully standardized multi-site trials, complying to EBM criteria, are still needed to bring the MRS assessment of brain tumors in general clinical practice.

In vivo ¹H MRS of prostate cancer

Functional imaging methods are much needed in the workup of patients suspected of prostate cancer, such as for detection of prostate cancer, local staging and metastasis, localization of cancer tissue in the prostate and treatment selection and assessment. In the detection of prostate cancer the analysis of ultrasound-guided biopsies from the prostate plays a major role, but due to sampling errors, often-negative biopsies occur despite a positive PSA level. Thus better imaging of the presence of cancer tissue would be desirable to guide biopsies. The stage of prostate cancer (occurrence of extra prostatic cancer) is often decisive in therapy-decision, but is mostly based on the so-called Partin tables of clinical findings valid for a general patient population and clearly the addition of a proper imaging examination might improve an individual assessment. The proper localization of cancer tissue in the prostate is also becoming of interest due to the introduction of new focal therapies such as intensity-modulated radiotherapy (IMRT) for local disease. Functional imaging to localize active tumor can be important for treatment assessment, and detection of recurrence. And finally it would be extremely important if a functional imaging method could predict the progression of localized prostate cancer to more aggressive variants. Among the potential MR methods that may contribute to solve these diagnostic questions ¹H MR spectroscopy is one of the promising candidates.

More than 10 years ago it was demonstrated that ¹H MR spectra of extracts of prostate tissue show a large number of metabolite signals, among which are signals for protons in citrate and choline compounds and that the (relative) signals for citrate may be decreased and that of choline compounds

increased in prostate cancer tissue [64, 65]. After the introduction of endorectal coils it also became possible to obtain in vivo ¹H MR spectra of small volumes of the prostate with sufficient signal to noise [66-68]. Fortunately the dominant peaks observed in these spectra are from protons in citrate, creatine and choline compounds. Compared to healthy peripheral or BPH tissue the signals of citrate were reduced and those of choline compounds often increased in cancer tissue and thus it was obvious that the tCho over citrate peaks could serve as a biomarker for prostate cancer. Weaker signals sometimes are also observed, for instance for protons in polyamines resonating in-between the creatine signal at about 3 ppm and the tCho peak at 3.2 ppm. In the interpretation of the data it is of note that citrate mainly occurs in the ducts of the prostate and hence a lower citrate may indicate altered metabolism as well as a reduction of luminal space in cancer tissue. Because tumor tissue can occur anywhere in the prostate it became clear that multi-voxel methods would be essential in further clinical studies. As the prostate is relatively small and embedded in adipose tissue this required the implementation of advanced RF pulses and sequences to suppress interfering strong lipid signals. In this way 2 and 3-dimensional MR spectroscopic imaging sequences have been realized [66, 69-71] of which 3D variants are now most favored as these can cover the whole prostate. Three-dimensional acquisitions can be performed in about 10-15 minutes at a spatial resolution down to about 0.4 cc, with sufficient signal to noise, at 1.5T. In the analysis of the data of patients it should be taken into account that different regions of the healthy prostate such as peripheral zone, central zone, and areas close to the urethra and the seminal vesicles have different amplitudes for citrate, creatine and "choline" compound signals. In addition MRS has to be performed sufficiently delayed with respect to the time of biopsy to avoid possible interference of a hemorrhage with spectral quality, due to magnetic field or morphological distortions.

Several studies were devoted to an evaluation of the clinical potential of 3D ¹H MRSI to localize cancer tissue in the peripheral zone of the prostate [72, 73]. After radical prostatectomy stepsection histopathology was performed and compared with the MRSI data in which the voxels were scored on a scale from malignant to benign using criteria based on the standard deviation of normal values. Because the signals of tCho and creatine are often not well resolved at 1.5T the common way of analyzing the data is by using the tCho plus creatine over citrate ratio, which ignores possible decreases in creatine in cancer tissue, but this can be taken into account in a refinement of the analysis. With sensitivities comparable to those obtained by standard T2 weighted MR imaging (about 70%), the specificity was significantly higher (up to about 85%) and by combining both methods sensitivity and specificity increased to about 90%. In a study that we recently completed on 34 patients using similar approaches, sensitivity and specificity in localizing prostate cancer both in the peripheral zone and central gland, by ¹H MRSI alone, was between 80 and 90% [74]. All these results are very promising, but the true clinical value can only be judged from results of multi-center trials. At this moment 2 trials are running that evaluate the properties of 3D ¹H MRSI to localize cancer tissue in the prostate: the International Multi-Centre Assessment of Prostate Spectroscopy (IMAPS) trial and a trial by the American College of Radiology Imaging Network (ACRIN 6659). Preliminary results of the IMAPS trial (7 clinical sites) show specificities and sensitivities comparable to single site studies for tumors in the peripheral zone and central gland [75, 76]. Potential confounding conditions in the localization of cancer in the prostate are other prostate diseases like prostatitus [77].

Although ¹H MRSI may be used to improve the reliability of diagnosing local staging, i.e. extracapsular extension [78], it is not expected to be the prime MR approach for this purpose, as the very high resolution MRI obtainable at 3T may be more suitable to address this problem [79].

MR spectroscopic imaging can also contribute to the planning and assessment of various treatments of prostate cancer and in the detection of recurrence after treatment e.g. [80-85]. Hormone deprivation is a common procedure in prostate cancer treatment and during this procedure MRSI shows a remarkable, partly selective, decrease in metabolite levels (metabolic atrophy) over time, which may point to important cancer features [86]. Metabolic atrophy also occurs upon radiation treatment, but much slower. A major clinical issue is the determination of the aggressiveness of prostate cancer and this is usually characterized by histology with the so-called Gleason score. It is of interest that the (tCho + cr)/citrate ratio is correlated with the Gleason score although the overlap of the current data precludes that it can be used for individual decisions [87, 88].

The clinical performance of MRSI of the prostate may be improved by still better shimming procedures (to decrease the number of low quality spectra), better signal to noise and better chemical

shift dispersion. With the rapid implementation of higher field systems (3T) in the clinic the prospects for improved clinical performance of MRSI of the prostate is very good provided that proper adaptations of the technology are implemented [89, 90].

<u>In vivo ¹H MRS of human breast cancer</u>

Currently the sensitivity for detecting invasive breast cancer by dynamic gadolinium contrast MRI is very high (up to 90% or higher), however its specificity may be rather low (down to 40%) and this is a major reason why ¹H MRS is being explored as a complementary MR tool to discriminate malignant from benign lesions in the breast. In addition ¹H MRS may have a unique role in therapy prediction and monitoring.

In vivo MRS of human breast cancer first was explored using the ³¹P nucleus [91], with typically high phosphomonoester and diester peaks present in spectra taken from cancer tissue. Spectra of extracts of this tissue showed that resonances from (glycero-)phosphoethanolamine and (glycero-)phosphocholine contributed to these peaks. In more recent years attention has switched to the ¹H nucleus, as it can be detected with a higher sensitivity allowing smaller tumors to be analyzed within shorter measurement times, and as no special hardware is needed it is rather easy to add to an MRI examination on a standard clinical system.

In the ¹H MR spectrum of the normal breast, without any signal suppression, the signals of lipids are dominating, but in tumors a more pronounced water signal appears. Although some clinical studies have used these signals in the assessment of breast cancer, e.g., [92], most investigations focus on the peak at about 3.2 ppm, characteristic for tumor tissue. Ex vivo high resolution NMR and so-called Magic Angle Spectroscopy studies of tissue biopsies showed that this peak mainly is composed of resonances from methyl protons in choline compounds such as choline, phosphocholine and glycerophosphocholine, e.g. see [93, 94] and commonly referred to as the total choline peak (tCho). In a number of studies the clinical value of detecting a peak at 3.2 ppm was explored to identify various breast carcinomas and benign lesions at the common clinical field strength of 1.5 T, e.g. see [95-98]. The tCho peak was also seen in spectra of normal breasts of lactating women, which has to be taken into account. The sensitivities and specificities reported in some of these studies to discriminate malignant from benign lesions were in the order of 80 to 85%. Although these initial in vivo ¹H MRS studies were promising they were hampered by some shortcomings. Artefactual signals may arise at the 3.2 ppm position due to spurious sidebands of large lipid signals. Most importantly the analysis in these studies was largely qualitative as usually only the visual absence or presence of a peak at 3.2 ppm in the spectrum was used as a decisive biomarker. However, variable MRS sensitivity due to different RF coil positioning or loading and field strength may interfere with choline signal visibility. The false positives often occurring in the in vivo studies indicated that a more robust and quantitative approach to assess the 3.2 ppm peak was needed. It was demonstrated that artefactual lipid sidebands can be overcome by specific pulse sequences such as TE averaging [99]. Quantitative approaches have been introduced, either using an external reference signal [100] or the internal signal of water as a reference [101]. Currently a quantitative approach appears to be essential as a tCho peak can also be detected in spectra of asymptotic breast tissue, especially at higher field strengths (> 1.5T) and as malignant breast tissue is characterized by a generally higher, but variable, tissue content of tCho compounds. The addition of quantitative ¹H MRS to dynamic contrast enhanced MRI has been shown to improve sensitivity, specificity, accuracy, and interobserver agreement between observer radiologists to discriminate malignant from benign breast lesions [102]. A more precise calibration of the resonance position of the tCho peak has also been proposed to separate benign tissue from malignant tissue in cases that a choline signal is detected [103].

Next to the use of ¹H MRS to improve the discrimination between malignant from benign breast cancer it may prove useful in the evaluation of treatments. This has been little explored yet, but an interesting example is a study where the change in the tCho signal was significantly different between patients with objective response and those with no response to a doxorubicin-based chemotherapy in locally advanced breast cancer [104]. In particular in these studies a quantitative approach is essential.

In current practice of ¹H MRS applied to breast cancer mostly single volume measurements are performed of one or several tumor locations in less than 10 minutes at a spatial resolution down to 1 cc. Positioning of the voxel at the proper tumor location is important, in particular in heterogeneous

tumors such as invasive ductal carcinomas [101]. Therefore a Gd enhanced MR image may be used for guidance as little effect of Gd on the tCho signal is anticipated. Recently also multivoxel spectroscopic imaging methods have been introduced, e.g. [105]. It remains to be evaluated how robust this can be performed in the presence of large lipid signals and optimal field shimming strategies are likely to be crucial for this purpose [106].

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